# THE EFFECT OF SUBMERGED PINE NEEDLES ON THE OVIPOSITION AND DEVELOPMENT OF ANOPHELES QUADRIMACULATUS SAY[1]

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## INTRODUCTION

A study of the effect of submerged pine needles on the oviposition and development of mosquitoes was instigated by a report from T. J. Roe, CWO, USN. Mr. Roe noticed that sand buckets filled with rain water and containing pine needles harbored no mosquito larvae, while those without needles produced several hundred larvae of *Culex quinquefasciatus* Say. It was hypothesized that the submerged pine needles could either cause the water to be unsuitable for oviposition, or to retard or inhibit larval development.

Various products from pine trees have been utilized as insect repellents and insecticides. Davis and Turner (1918) found that pine sawdust had a decidedly repellent effect on cutworms. Bishopp et al (1923) noted that various pine oils have a definite repellent value against flies, and Cory (1928) stated that volatile emanations from crude pine oil alone and in emulsions were markedly repellent to ants. Pine oil possesses definite repellent value against certain *Aedes* mosquitoes according to MacNay (1939), although not as effective as other materials tested. Studies by Blagoveschenskii and coworkers (1943) on d-x-pinene, obtained as a waste product in the process of purification of juniper oil in Central Asia, indicated that it was repellent to mosquitoes. With reference to insecticidal properties of pine tree products, Headlee (1929) found one of the fractions obtained by the de-

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structive distillation of pine stumps, roots and branches with caustic soda to be effective in trunk treatments to kill overwintering codling moth larvae. Barnes (1925) showed that "pine oil, though very toxic to mosquito larvae is not suitable for use on water, as the film it forms breaks up too rapidly for practical purposes." He concluded that pine oil has a powerful soporific or paralyzing effect upon the larvae and pupae and results in their death either directly or apparently by drowning while under the effects of the drug. According to Shugar and Wyrap (1942) and Shesterikova and Bushurova (1942) extracts from the distillation of pine wood are toxic to mosquito larvae.

Very little is to be found in the literature about the insecticidal properties of pine needles. Lagereva (1947) discussed the freeing of cattle of *Psoroptes* by rubbing the affected parts with a liniment prepared from pine needles and crude cresol. Barnes (1925) found that the eggs of *Anopheles quadrimaculatus* are not uniformly destroyed by the film formed by pine oil and kerosene.

With reference to the composition of pine oil, Wirth (1943) stated that it is obtained by the steam distillation of the wood of *Pinus palustris* Miller and other species of *Pinus*. It is a volatile oil composed chiefly of tertiary and secondary terpene alcohols. Pearson (1935) gives its composition as mainly terpenes, alcohols, ethers and ketones. According to Bau (1921) the residue left by steam distillation of pine needles is a wax containing cetyl, ceryl and myricly alcohols; palmitic, phdroxypalmitic (A) and steric acids; phytesterol; obeitic acid; dihydroxystearic acid and lower fatty acids, including butyric. Working with Philippine pine needle oil from *Pinus insularis* de Santos, West and Esquerra (1931) gave an analysis of the oil as largely *A* and *B* pinene and a small percent of esters calculated as bornyl acetate.

## MATERIALS

Tests with submerged pine needles were conducted with *Anopheles quadrimaculatus* Say. eggs, larvae, pupae and adults. The needles were from large Loblolly pine trees, *Pinus taeda* L. Oviposition studies were conducted by placing pine needles in Petri dishes containing tap water in cages with adult female mosquitoes. Larval and pupal studies were conducted in 250 ml. beakers and 8 by 12 inch stainless steel pans. The larvae were fed measured amounts of ground commercial dog food. All experiments were carried on at room temperature 76–80° F. The pine needles averaged seven inches in length and included the encircling sheath at the base (groups of three needles).

## PROCEDURE

Oviposition preference was determined by placing 10, 20, and 30 pine needles in separate Petri dishes containing tap water. The needles were cut into approximately one-fourth inch lengths. The three dishes were placed three inches apart in a line across the center of the floor of a 19 by 19 inch screened cage containing approximately 500 adult *Anopheles quadrimaculatus*. Three dishes containing tap water as controls were placed individually in front and against each of the dishes containing the needles also three inches apart. All dishes were left overnight in the cage and removed after 21 hours. The results are shown in Table 1.

TABLE 1

EFFECT OF SUBMERGED PINE NEEDLES ON OVIPOSITION AND EARLY LARVAL DEVELOPMENT OF *Anopheles quadrimaculatus*

| Number of needles | Number of eggs | Number hatched | Per cent hatched | Mortality of larvae age in days | | | | | Per cent dead |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | |
| 10 | 259 | 206 | 79.5 | 142 | 184 | 198 | 204 | 206 | 100 |
| Control | 4,155 | 3,812 | 91.7 | | 58 | 81 | 150 | 160 | 4.2 |
| 20 | 313 | 200 | 63.9 | 143 | 192 | 200 | | | 100 |
| Control | 1,607 | 1,470 | 91.5 | | 44 | 61 | | | 4.1 |
| 30 | 204 | 68 | 33.3 | 57 | 68 | | | | 100 |
| Control | 3,086 | 2,831 | 91.7 | | 50 | | | | 1.8 |

Collectively, the three dishes containing pine needles contained 776 eggs compared with 8,848 in the controls or only eight per cent of the eggs were deposited on the pine needle water. However, subsequent tests involving 30 pine needles indicated that the mosquitoes laid as many eggs in the pine needle water as they did in the controls. The number of eggs laid on the water in the three dishes containing needles were not appreciably different. An average of 61 per cent of the eggs laid in the pine needle water ultimately hatched, compared with 92 per cent in the controls. In individual cases, the percentage of eggs that hatched decreased from 80 per cent in the dish with 10 pine needles to 33 in the one with 30 needles, while the controls remained constant at 92 per cent hatch. Apparently, the eggs that were laid during the first few hours were deposited on the water before the wax film was completely formed. Those deposited later were on top of the film, and although slightly moist, did not hatch. This would account for the decreased percentages of hatch as the number of needles increased, forming a film more rapidly and of greater density. The number of days to complete larval mortality decreased from five days for 10 needles to two for 30 needles, indicating a more rapid death rate with increased numbers of needles.

In preliminary tests with older larvae, a large number of green and dry (fallen) pine needles (300 each, 7 inches long) were cut into one-half inch lengths, ground separately in 200 ml. tap water and sand with mortar and pestle, and the supernatant liquid was placed in 250 ml. beakers. Fifty larvae 2 days old were placed in each beaker. Those in the liquid from green pine needles showed 68 per cent apparently dead in 2 hours and those in the liquid from dry needles, 50 per cent. In five hours, the green needle-water produced 72 per cent mortality while the dry needle water remained at the 50 per cent level. In twenty-one hours (over night) all were dead in both groups while the controls attained only 15 per cent mortality. Subsequent tests on the basis of whole green and dry needles indicated that the green needles produced greater mortality in 48 hours than the dry. For this reason all further observations were conducted with green needles. The results for green needles are given in Table 2.

In order to determine the number of needles necessary to produce mortality in five day old larvae, 5, 10, 20, 30, 40, 50 needles cut into half inch lengths were placed in separate 250 ml. beakers containing 200 ml. of water. Fifty larvae were placed in each beaker. The larvae were fed measured amounts of ground dog food. As shown in Table 2, ninety-six to ninety-eight per cent mortality was produced in nine days with 30 to 40 needles respectively. Fifty needles yielded 100 per cent mortality in five

TABLE 2

MORTALITY OF LARVAE EXPOSED TO SUBMERGED GREEN PINE NEEDLES

| Number needles | Length of cut needles | Type container | Volume water | No. larvae | Mortality in days | | | | | | | | | Per-cent dead |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 5 | 0.5 inch | 250 ml. beaker | 200 ml. | 50 | 1 | 6 | 10 | 10 | 12 | 15 | 19 | 19 | 19 | 38 |
| 10 | " | " | " | " | 9 | 9 | 9 | 9 | 13 | 18 | 21 | 23 | 23 | 46 |
| 20 | " | " | " | " | 13 | 13 | 13 | 13 | 13 | 21 | 23 | 23 | 23 | 46 |
| 30 | " | " | " | " | 22 | 22 | 25 | 26 | 33 | 41 | 43 | 47 | 48 | 96 |
| 40 | " | " | " | " | 25 | 38 | 38 | 45 | 46 | 48 | 49 | 49 | 49 | 98 |
| 50 | " | " | " | " | 32 | 40 | 41 | 46 | 50 | | | | | 100 |
| Control | — | " | " | " | 0 | 4 | 5 | 5 | 7 | 7 | 7 | 7 | 7 | 14 |
| 30 | 0.25 inch | " | " | " | 48 | 48 | 48 | 49 | 50 | | | | | 100 |
| 50 | " | " | " | " | 49 | 50 | | | | | | | | 100 |
| 70 | " | " | " | " | 41 | 50 | | | | | | | | 100 |
| 100 | " | " | " | " | 50 | | | | | | | | | 100 |
| Control | — | " | " | " | 0 | 0 | 4 | 4 | 10 | | | | | 20 |
| 100 | 0.25 inch | 8″ × 12″ pan | 800 ml. | 100 | 58 | 71 | 84 | 90 | 92 | 92 | 98 | 100 | | 100 |
| 200 | " | " | " | 200 | 104 | 156 | 180 | 196 | 197 | 199 | 200 | | | 100 |
| 300 | " | " | " | 200 | 110 | 160 | 183 | 199 | 200 | | | | | 100 |
| Control | — | " | " | 200 | 2 | 12 | 12 | 12 | 12 | 12 | 14 | 21 | | 11 |

days. Fourteen per cent mortality occurred in the controls. Seven day old larvae exposed to 30, 50, 70 and 100 one-fourth inch needles under the same conditions reached 100 per cent mortality in five, two, two and one day respectively with 20 per cent mortality in the controls after five days.

Since needles cut into one-fourth inch lengths produced mortality in a shorter time than those one-half inch long, tests were made in $8 \times 12$ inch stainless steel pans containing 800 ml. of water to determine the mortality over a larger surface area. One hundred to 200 five day old larvae were used per pan. The needles in one-fourth inch lengths were placed in the pans at the rates of 100, 200, and 300 needles per pan. The mortality reached 100 per cent in eight, seven and five days respectively with 11 per cent dead in the controls. Fifty-two pupae developed in the controls, but none developed in the pans containing pine needles.

The results indicate that *Anopheles quadrimaculatus* larvae in 800 ml. of water with a surface area of 96 square inches do not reach maturity in the presence of 100 pine needles under laboratory conditions, and that the time necessary for death decreases as the number of needles is increased.

The needles produce a wax-like film over the surface of the water which is similar to a coating of very thin paraffin. The actions of the larvae in attempting to remain at the surface of the water, indicate that drowning may play a part in causing death, although the decrease in egg hatch may indicate a toxic action also. Oviposition will occur on water saturated with turpentine, but very few eggs hatch. In very dilute solutions of turpentine, the eggs hatch, but the larvae die shortly thereafter. It is possible, therefore, that the terpene alcohols may be a prominent factor in larval mortality. Starvation may also be a factor since the larvae were unable to feed at the surface and food would not spread over the surface.

In order to determine the effect of pine cones on larval development, 15 young cones one inch long by one-fourth inch in diameter were placed in 200 ml. of water in a 250 ml. beaker with 50 larvae. Ninety-six per cent of the larvae were dead in four days.

Pine gum in water did not cause mortality to larvae.

One-half inch lengths of one-fourth inch diameter limbs (from section of limb eight inches long producing pine needles) gave 28 per cent mortality to 50 larvae in 200 ml. of water (250 ml. beaker) in 48 hours and only 66 per cent in 11 days.

Pupal mortality was determined by placing 100 pupae in 800 ml. of tap water in each of three 8 by 12 inch pans containing 100, 200 and 300 submerged green pine needles respectively. The needles were cut into approximately one-fourth inch lengths. The pupae were from larvae that had pupated within 24 hours of the experiment. The control consisted of 100 pupae in the same type pan in tap water. Mortality counts were made 24, 48, and 72 hours following initiation of the test. The results are given in Table 3.

TABLE 3

The Effect of Submerged Pine Needles on Pupal Mortality and Adult Emergence

| Number of needles | Number of pupae | Percent pupal mortality by days | | | Percent adults from remaining pupae | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 day | 2 days | 3 days |
| 100 | 100 | 7 | 24* | 25* | .01 | 86.8 | 100 |
| 200 | 100 | 8 | 15** | 19* | .00 | 63.5* | 100 |
| 300 | 100 | 5 | 21* | 26* | .01 | 56.9* | 100 |
| Control | 100 | 4 | 7 | 7 | .01 | 82.8 | 100 |

* Significant at the .001 percent level of confidence (Significance of difference between proportions).

** Significant at the .01 percent level of confidence.

The pupal mortality in each of the pans containing submerged needles was significantly greater than the mortality in the control pan on the second and third days. On the second day the adult emergence from the pan containing 100 needles was not significantly greater than the controls, but the emergence from the pans containing 200 and 300 needles was significantly less than the emergence from the 100 needle and control pans. This indicates that submerged pine needles cause some increase in mortality and retard adult emergence. However, 100 per cent of the remaining pupae had produced adults by the third day in all cases.

In order to observe the effect of pine needle water on adult mosquitoes, 290 green needles were boiled for 30 minutes in a loosely capped beaker. Twenty adult females were sprayed with the supernatant fluid with an atomizer until the insects were wet. There was no mortality in 24 hours. Similarly 20 females sprayed with supernatant fluid from uncut needles allowed to stand in water 24 hours produced no mortality. Material from green pine needles cut into one-half inch lengths and ground in water with sand produced no mortality when sprayed on adult females. The controls were sprayed with tap water and remained normal. Organic solvent extraction might help clarify the question of insecticidal activity against adult mosquitoes and the mode of action on larval forms since the material released by the needles is relatively insoluble in water.

## CONCLUSIONS

*Anopheles quadrimaculatus* females exhibit no aversion to oviposition on water containing green pine needles. The needles produce a wax-like cover over the surface of the water. The number of eggs that hatch decrease as the number of pine needles in the water is increased. The death rate of the larvae increases as the number of pine needles becomes greater. The larvae are killed in water containing either green or dry needles, but greater mortality is produced by the green needles. The detrimental action of needles is enhanced by cutting them into one-fourth inch lengths. One hundred needles are required to kill larvae in nine days in 800 ml. of water with a surface area of 96 square inches. The time is reduced to five days when 300 needles are utilized.

Since egg hatch and larval mortality are adversely affected by dilute solutions of turpentine, it is possible that terpene alcohols in the pine needles may be a prominent factor in larval mortality. Drowning, due to the film over the water, and starvation, due to inability to obtain surface food, may also contribute to mortality.

Young pine cones have been shown to inhibit larval development. Small branches cut into short lengths show some detrimental effect on larvae. Pine gum did not cause larval mortality.

Submerged pine needles caused some pupal mortality on the second and third days, and retarded emergence on the second day. However, all remaining pupae emerged on the third day.

There is no evidence that the water from submerged pine needles is toxic when sprayed on adult mosquitoes. Organic solvent extracts might clarify the question of insecticidal activity since the material released by the needles is relatively insoluble in water.

Field tests of this method of mosquito control are warranted, and it appears to provide an alternate method of field control under certain conditions. However, rain, wind, and other factors could conceivably nullify the effect of the wax film on the water.

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